



BRIEF COMMUNICATIONS

Yeast Two-Hybrid Interactions in B Lymphocytes and Cardiac Myocytes

Fraser I, Pearman T, Williams B, Sinkovits RS, Dufford M, Lin K-M, Hsueh R, Yan Z, Grosseohme J, Pierce R, Polasek J, Girouard J, Heichman K, Bartel P, Sambrano GR

Alliance for Cellular Signaling Laboratories, California Institute of Technology, Pasadena, CA; University of California, San Diego, CA; UT Southwestern Medical Center, Dallas, TX; University of California, San Francisco, CA; and Myriad Genetics Inc., Salt Lake City, UT

Abstract: The Alliance for Cellular Signaling (AfCS) is using the yeast two-hybrid system in an ongoing project to identify protein-protein interactions that compose the large network of signal transduction molecules in murine B lymphocytes (B cells) and cardiac myocytes. Proteins known or suspected to transduce signals in these cell types have been selected as baits and screened against specific activation domain libraries prepared from B-cell and myocyte sources. So far, we have identified many novel protein-protein associations in addition to a reassuring subset of interactions previously reported in the literature. Some of the observed interactions occur between proteins already known to participate within the same signaling pathway but not previously shown to directly interact. Other interactions suggest the possibility of crosstalk between unrelated pathways or the participation of novel proteins within established signaling cascades. We present an example of this interaction data for proteins involved in signaling pathways downstream of the B-cell receptor.



[Introduction](#)

[Library Prep](#)

[Bait Selection](#)

[Bait Design](#)

[Project Status](#)

[References](#)

[Contributors](#)

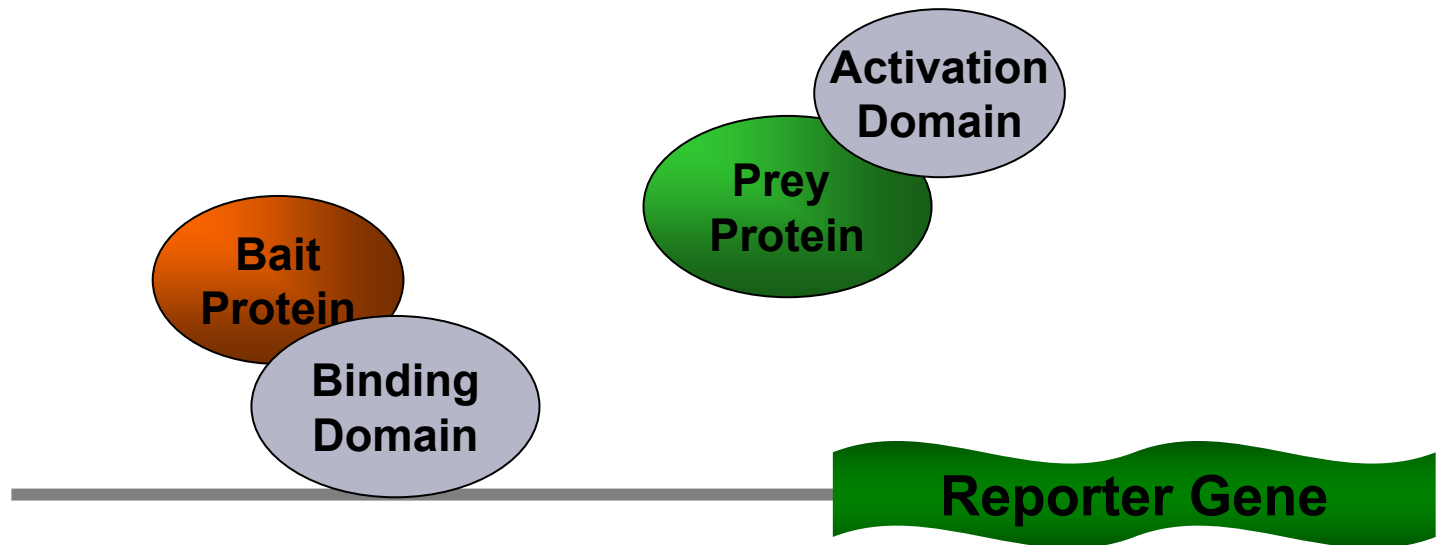
Introduction

Several approaches are being taken to examine the complexity of the signal transduction networks in B lymphocytes (B cells) and cardiac myocytes. In addition to characterizing the breadth of signaling responses in these cells, the Alliance for Cellular Signaling (AfCS) is identifying the components that participate in each of the networks and the interactions that occur among their respective components. Identification of intracellular protein-protein interactions and the conditions that affect change in such interactions are central to understanding the mechanisms of signal transduction.

The AfCS is utilizing high-throughput methods to detect interactions among signaling molecules expressed in B cells and cardiac myocytes. In an effort to produce a large body of information on protein-protein interactions in these cells, the AfCS has established a collaboration with Myriad Genetics (<http://www.myriad.com>) to perform large-scale yeast two-hybrid screens.

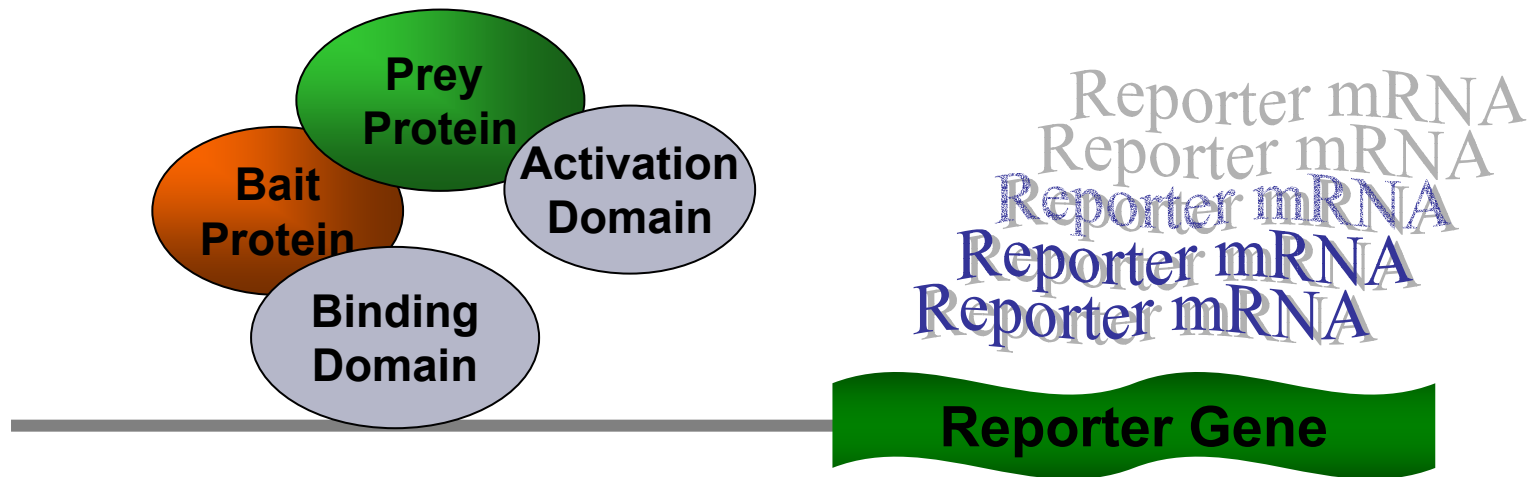
The Yeast Two-Hybrid System

- The yeast two-hybrid (Y2H) system is a well-established method (1,2) that allows for the rapid identification of binary interactions between a chosen test protein (the bait) and an interacting protein (the prey).
- This is achieved by expressing the bait protein as a hybrid, fused to the DNA binding domain of a reporter gene, and screening it against a library of prey candidates, which are fused to the corresponding transcriptional activation domain of the reporter gene.



The Yeast Two-Hybrid System (cont.)

- Both fusion proteins are expressed in a yeast cell where the reporter gene expression is under the control of binding sites for the DNA-binding domain.
- Interaction of bait and prey proteins localizes the activation domain to the reporter gene, causing it to be expressed. Since the reporter gene typically codes for a survival factor, yeast colonies will grow only when an interaction occurs.



Preparation of Cell-Specific Libraries

- Screening of an activation domain library of proteins that are representative of a specific cell type may permit the identification of cell type-specific protein-protein interactions.
- Total RNA was purified from the sources shown below using the Tripure reagent (as described in protocols [PP00000009](#); [PP00000133](#)), and polyA⁺ mRNA was isolated from total RNA using the PolyA Tract mRNA Isolation System III ([Promega Protocol](#)).
- Since a number of proteins that play a central role in B-cell signaling pathways may be expressed at low levels in resting cells, mRNA was also isolated from B cells stimulated with a cocktail of ligands selected to activate the major B-cell signaling pathways.

Table 1. *Sources of mRNA for Y2H libraries.*

B-Cell mRNA Sources	Cardiac Myocyte mRNA Sources
Murine primary resting B cells	Murine primary cardiac myocytes
Murine primary B cells stimulated with a ligand cocktail of anti-IgM, anti-CD40, and IL-4 for 4 hr	Nk-TAg murine cardiac myocyte cell line (3)
WEHI-231 murine B-cell line	

Preparation of Cell-Specific Libraries (cont.)

- An activation domain library was prepared by Myriad Genetics from each of the mRNA preparations using a proprietary genetic technique that reduces false positives (such as structural, heat shock, mitochondrial, and ribosomal proteins) from the libraries. After preparation, the libraries were validated and analyzed for complexity and average clone size (see Table 2, below).
- The B-cell-specific libraries all passed quality control standards (titer, complexity, insert size, mating efficiency, and sequencing ability) and were therefore combined in equal ratios to produce a single B-cell library for the AfCS yeast two-hybrid screens.
- However, the myocyte libraries were not combined due to a high proportion of mitochondrial DNA in the primary myocyte preparation. The myocyte libraries were therefore kept separate in the AfCS yeast two-hybrid screens and analyzed as “Primary cardiac myocytes” or “Nk-TAg myocyte cell line”(3).

Table 2. *Complexity and average clone size of each library.*

Library	Complexity	Av. Clone Size
Resting primary B cells	1.60E + 07	0.95 Kb
Stimulated primary B cells	4.30E + 07	1.00 Kb
WEHI-231 B-cell line	5.70E + 07	1.00 Kb
Primary cardiac myocytes	4.00E + 06 (80% mitochondrial)	0.80 Kb
Nk-TAg myocyte cell line	3.90E + 07	1.10 Kb

Bait Selection

- The goal of high-throughput Y2H screening is to identify as many possible protein interactions that might occur in the cells of interest. Given a projected screening capacity of 100 signaling proteins per year, the selection of candidate proteins is not simply arbitrary. A number of criteria were taken into consideration in the selection of bait protein candidates:
 - Proteins known to be expressed in B cells and/or cardiac myocytes.
 - Proteins known or suspected to be involved in signaling pathways in B cells and/or cardiac myocytes.
 - Proteins believed important in GPCR or BCR signaling leading to PIP3 generation. These were selected first in an attempt to define interactions within a focused network of signaling molecules.
- Preference was also given to proteins that had a higher likelihood of success in the yeast two-hybrid assay, specifically, proteins with well-defined modular domains exhibiting secondary structure suggestive of involvement in protein-protein interactions.

Introduction

Library Prep

Bait Selection

Bait Design

Project Status

References

Contributors



Bait Design Principals

- Myriad Genetics has determined, through extensive experience with the yeast two-hybrid system, that fragments of proteins representing folded domains are often more effective than the full-length protein in identifying physiologically relevant interactions in this assay. This approach is especially important when attempting to identify binding partners for membrane proteins, because the interactions take place in the yeast nucleus. The expression of either cytosolic or extracellular domains of membrane proteins has proven to be effective.
- The following general criteria were followed for bait design:
 - Small protein fragments are generally most effective, so baits were designed to be 100 to 400 amino acids.
 - If the domain structure of a given bait protein was already established, the specific baits were designed to represent one or more folded domains.
 - For cases in which domain structure was not available, a variety of secondary structure prediction algorithms were used to predict domains and thus direct bait design.
 - Baits were designed to cover the entire protein, with several overlapping fragments, as not all baits will work effectively.
 - Signal sequences were avoided in secreted and transmembrane proteins.
 - Transmembrane domains were avoided in membrane proteins.

Introduction

Library Prep

Bait Selection

Bait Design

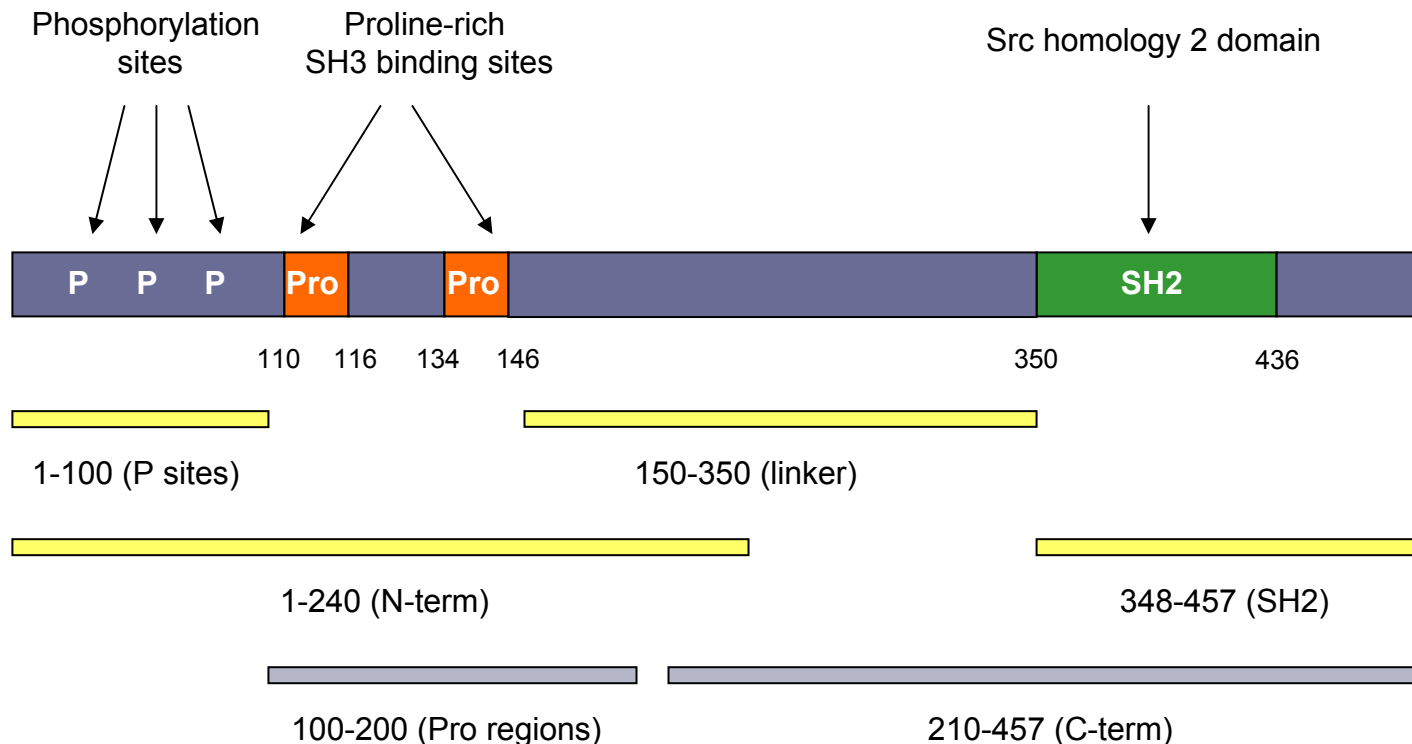
Project Status

References

Contributors



Bait Design Example: BLNK



B-cell linker protein (BLNK) is an adapter protein that interfaces B-cell receptor tyrosine kinases to several downstream effector molecules via its SH2 and SH3 domains (4). Baits derived from this protein are shown above to illustrate the general design principals. Interactions have been identified for the baits highlighted in yellow (see [interaction data](#)).

Project Status

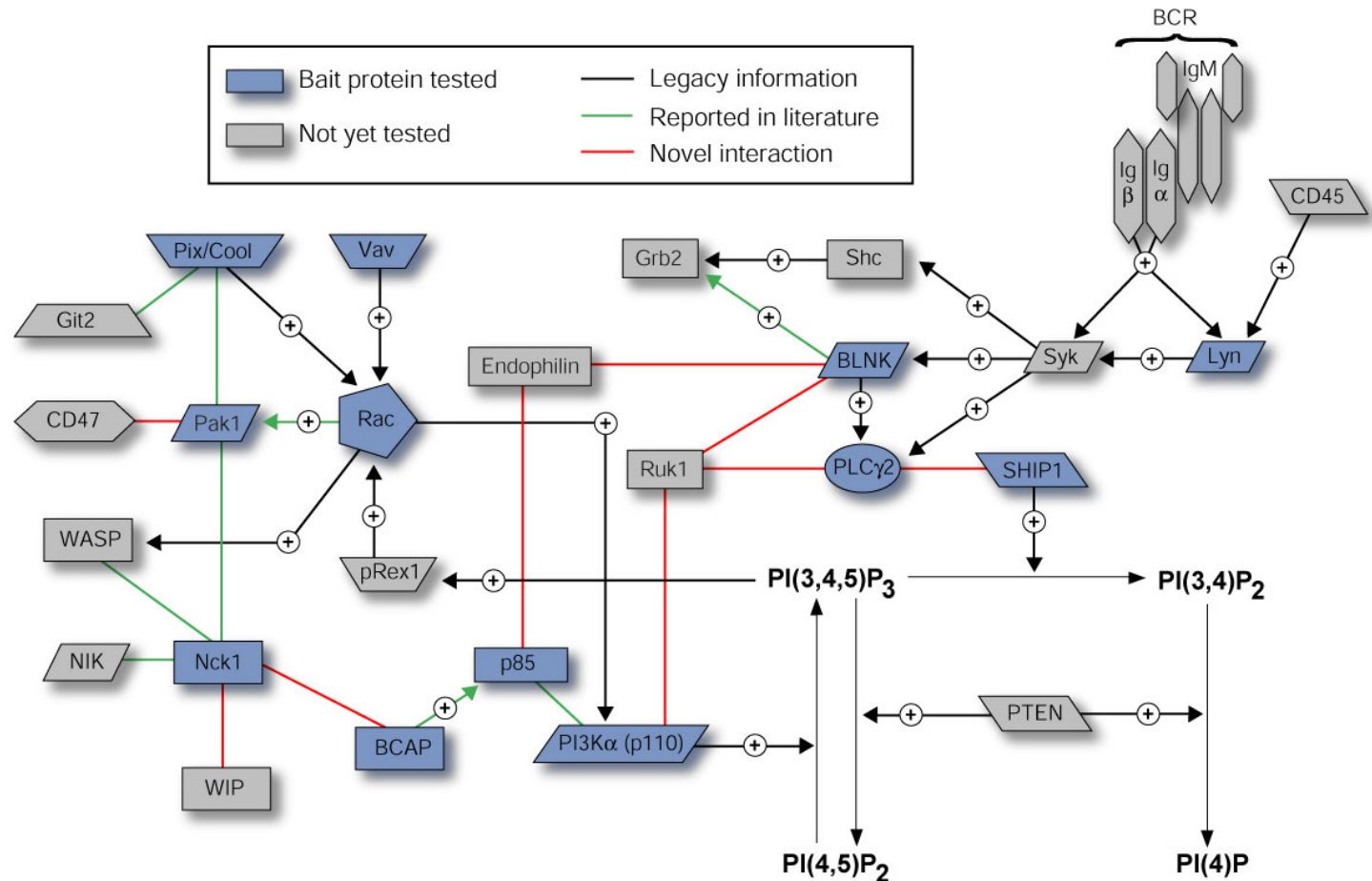
Although the screening effort is still in an early phase, we have observed interactions among proteins that are known or suspected to participate within the same network of signaling pathways. Table 3, shown on the next page, lists several interactions that may function in the PIP3 network of molecules in B cells. Using selected data, one can begin to build interaction maps such as the one shown on page 12. As interaction data continues to amass, the development of such maps will become more informative and provide us with important clues regarding the topology of the cellular signaling network in these cells.

- As of (April 2, 2003), 154 proteins have been selected for screening ([see interactions database](#)).
- Interaction data is available for 54 of these bait proteins.
- 404 bait-prey interactions have been identified.
- We expect to screen approximately 100 bait proteins per year.

Table 3. *Selected Y2H Data.*

Bait Protein	Prey Protein	Interaction Reported in Literature?
PI3 kinase p110 alpha	PI3 kinase p85	Y
PI3 kinase p110 alpha	PI3 kinase p55	Y
PI3 kinase p110 alpha	Ruk1	N
Phospholipase C gamma 2	Ruk1	N
Phospholipase C gamma 2	SHIP	N
BLNK	Grb2	Y
BLNK	Ruk1	N
BLNK	Endophilin	N
PI3 kinase p85	Endophilin	N
Pak1	Rac1	Y
Pak1	Cool1	Y
Pak1	Nck1	Y
Pak1	CD47	N
Nck1	NIK	Y
Nck1	WASP	Y
Nck1	WIP	N
Nck1	BCAP	N

Signaling Protein Interaction Map



Protein interactions identified by yeast-two hybrid screening (green and red lines) using selected bait proteins (blue shapes) are shown among B-cell receptor signaling pathway components.

References

1. Fields S and Song O. (1989) Nature 340(6230), 245-246. [\[PubMed\]](#)
2. Chien CT, Bartel PL, Sternglanz R, and Fields S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88(21), 9578-9582. [\[PubMed\]](#)
3. Rybkin II, Markham DW, Yan Z, Bassel-Duby R, Williams RS, and Olson EN. (2003) J. Biol. Chem. (epub ahead of print). [\[PubMed\]](#)
4. Fu C, Turck CW, Kurosaki T, and Chan AC. (1998) Immunity 9(1), 93-103. [\[PubMed\]](#)

Authors*

Concept, Design, & Supervision

[Iain Fraser](#)^{†‡}

Terrece Pearman[§]

Brandi Williams[§]

Gilberto R. Sambrano^{||}

Karen Heichman[§]

Paul Bartel[§]

Material

Robert Hsueh[¶]

Keng-Mean Lin[¶]

Zhen Yan[¶]

Data Collection and/or Processing

Terrece Pearman[§]

Brandi Williams[§]

Robert S. Sinkovits[#]

Max Dufford[§]

Technical Assistance

Jody Girouard[¶]

Joella Grossoehme[¶]

Read Pierce[¶]

Jason Polasek[¶]

Editors

Ashley K. Butler

Duke University, Durham, NC

[Gilberto R. Sambrano](#)^{**}

University of California San Francisco, San Francisco, CA

Reviewers

James T. Stull

UT Southwestern Medical Center, Dallas, TX

David Fruman

University of California Irvine, Irvine, CA

* Please refer to the AfCS policy on [authorship](#).

† To whom scientific correspondence should be addressed.

‡ AfCS Molecular Biology Lab, California Institute of Technology, Pasadena, CA.

§ Myriad Genetics Inc., Salt Lake City, UT.

|| University of California San Francisco, San Francisco, CA.

¶ AfCS Cell Preparation and Analysis Lab, UT Southwestern Medical Center, Dallas, TX.

AfCS Bioinformatics Lab, University of California San Diego, La Jolla, CA.

** To whom questions or comments about the *AfCS Communications* should be addressed.